

# Development of a medium throughput electrophysiology assay to investigate state-dependency of sodium channel blockers using QPatch

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## INTRODUCTION

Various members of the voltage-gated sodium channel family are of interest as potential therapeutic targets (e.g. Nav1.7 and Nav1.8), others pose significant safety risks should compounds block their activity (e.g. Nav1.5 in the heart). The identification of isoform-specific sodium channel modulators is therefore of great importance in drug discovery.

The "gold standard" for functional assessment of ion channel modulators remains the manual patch clamp technique, but these studies are labour intensive and low throughput so are not suitable for screening large numbers of compounds. The automated electrophysiology platform, QPatch, is capable of producing data of equivalent quality to that obtained using manual patch, but at significantly higher throughput (up to 16 cells can be patched in parallel). We therefore aimed to develop an assay to enable the production of high quality pharmacological data, without compromising on quality.

Assay development has focussed on the Nav1.5/CHO cell line, but the fundamental principles should apply to other Nav1.x family members.

## AIMS

- To evaluate the suitability of the CHO ChanClone™ Nav1.5 cell line over-expressing recombinant human Nav1.5 for use on the automated electrophysiology platform, QPatch
- To develop an assay suitable for the investigation of pharmacological modulators of Nav1.5 channels using QPatch
- To obtain the maximum amount of information from each experimental run, thereby reducing cost per data point

## METHODS

**Cell line construction:** A plasmid encoding the human sodium channel Nav1.5 (NM001099405) was stably integrated into the CHO cell line. Monoclonal populations were screened in functional expression tests. Clones exhibiting activity were then selected based on sealing and current amplitude. This CHO ChanClone™ Nav1.5 cell line is optimised for automated patch-clamping with above 80% success rates

**Cell preparation:** Adherent CHO ChanClone™ Nav1.5 cells were maintained in culture as per instructions from Genionics. A cell suspension was centrifuged and resuspended in extracellular recording solution on the QPatch platform and then applied to the QPatch16 planar electrode plate. 16 whole cell voltage clamp recordings were performed in parallel at room temperature on QPlate16.

**Electrophysiology:** Individual voltage protocols are described in the appropriate sections. Data was acquired using a Windows-based computer running QPatch Assay Software v3. Series resistance error was compensated by approximately 100%. A junction potential of +8.8 mV was not corrected for.

The extracellular solution contained (mM) 105 choline chloride, 35 NaCl, 3 KCl, 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 10 HEPES, 10 glucose (pH 7.2, osmolality 295-305 mOsm), whilst the intracellular solution contained (mM) 140 CsF, 10 NaCl, 1 EGTA, 10 HEPES (pH 7.2, 285-296 mOsm).

**Compounds:** Amitriptyline and flecainide (Sigma) were prepared in glass vials in DMSO to a 1000X final concentration stock. Tetrodotoxin (Tocris) and lidocaine were prepared in extracellular solution. All compound dilutions were adjusted to contain an identical concentration of vehicle (0.3% DMSO). Compounds were plated in glass 96-well plates.

**Data Analysis:** Recorded currents were analyzed using QPatch Assay Software v3 (Sophion, Denmark). Data were then exported into Excel or GraphPad Prism for further analysis where appropriate.

To determine stability of responses, the peak current amplitude recorded at -20 mV was plotted against time for each cell. Data were normalised to the first response obtained in any given cell. Sigmoidal IC<sub>50</sub> curve functions were fitted using the equation:  $y = 100 / (1 + [x / IC_{50}]^h)$ . Where IC<sub>50</sub> is the concentration by which 50% of the current is blocked and h is Hill slope.

## RESULTS

### The CHO ChanClone™ Nav1.5 cell line functions well on QPatch and currents resemble those described in the literature

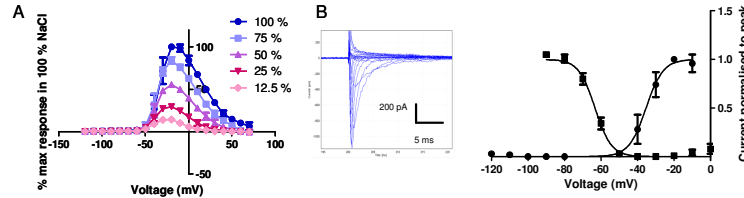
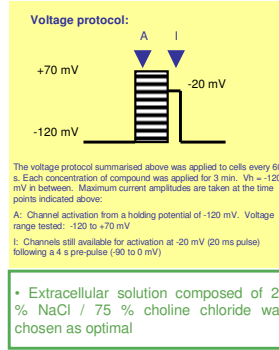


Fig 1. Current amplitudes were large so I-V relationships were plotted in reduced Na<sup>+</sup> extracellular buffers to determine optimal conditions (A). Data are mean ± SEM from n = 3 experiments. A representative trace from a single cell showing currents in response to voltage pulses described in box 1 in 25% NaCl solution is shown (B).

Fig 2. The CHO cell line stably expressing human Nav1.5 was characterised using QPatch. V<sub>1/2</sub> for activation was calculated as -35.3 ± 0.7 mV (n = 4; mean ± SEM) and V<sub>1/2</sub> for inactivation was calculated as -63.2 ± 0.7 mV (n = 8)



### QPatch enables the rapid characterisation of pharmacological modulators e.g. Tetrodotoxin

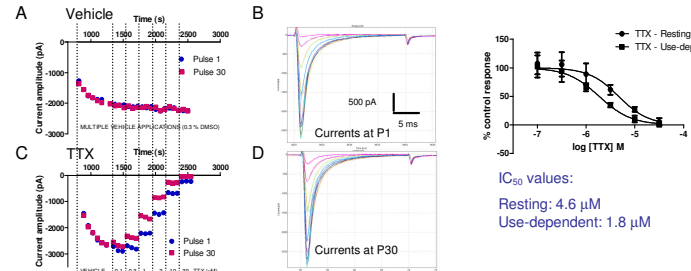


Fig 3. Current amplitudes in the presence of vehicle (0.3% DMSO) were stable over time (A). Increasing concentrations of TTX inhibited Nav1.5-mediated currents (C) with effects being more pronounced at P30 (pink – see yellow box) than P1 (blue). Raw traces of currents obtained at P1 (B) and P30 (D) are shown.

Fig 4. Concentration-effect curves for TTX acting at the resting state, inactivated state and in a use-dependent manner are shown. Data are mean ± SEM from n = 5 experiments.

### Multi-pulse assay maximises data output and efficiency to produce 3 IC<sub>50</sub>s per cell and at least 180 data points per experiment (QPlate)

- The multi-pulse assay allows IC<sub>50</sub>s to be calculated based on resting state, inactivated state and use-dependent block from each individual cell.
- The data shown in Fig 5 are from one experiment. In this example, from a single experimental run using one QPlate, 10 cells completed the full protocol = 30 IC<sub>50</sub>s from 4 compounds (180 data points).
- Vehicle controls (0.3% DMSO) were stable at each time point (P1, P2, P30)
- IC<sub>50</sub>s calculated are in good agreement with those obtained using simpler voltage protocols and with the published literature

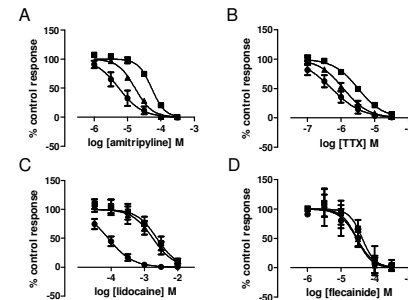


Fig 5. Concentration-effect curves for Amitriptyline (A), TTX (B), lidocaine (C) and flecainide (D) acting at the resting state, inactivated state and in a use-dependent manner are shown. Data are mean ± SEM from n = 2-3 experiments (see Table 1 for IC<sub>50</sub>s and more details).

## SUMMARY

TABLE 1: A summary of data obtained from a single experimental run using the multi-pulse protocol and compared to published literature data. Duplicate or triplicate data points are highly reproducible and correlate well with in house and literature data so are suitable for compound screening.

		Amitriptyline	Lidocaine	TTX	Flecainide
<b>Inactivated</b>	IC50	3.1 μM	133.0 μM	0.61 μM	28.2 μM
	Hill	1.26	1.54	1	2
	N=	2	3	3	2
<b>Resting</b>	IC50	52.8 μM	2.8 mM	2.9 μM	37.6 μM
	Hill	1.6	1.05	1.03	3.7
	N=	2	3	3	2
<b>Use-dep</b>	IC50	15.4 μM	2.0 mM	1.02 μM	26.6 μM
	Hill	1.4	1.05	1.02	1.85
	N=	2	3	3	2
<b>Literature</b>	IC50	0.58 μM <sup>4</sup>	318 μM <sup>1</sup>	1.3 μM <sup>3</sup>	11.0 μM <sup>5</sup>
<b>IW data<sup>2</sup></b>	IC50	1.0 μM	102 μM	0.4 μM	6.5 μM

Abbreviations: Hill: Hill slope; N: number of cells tested; Literature: data published based on manual patch clamp experiments investigating inactivated state block; IW data: published data based on IonWorks experiments.

## CONCLUSION

- The assay described provides reproducible, high quality assessment of compound potency and state-dependency at the human Nav1.5 channel
- The multi-state protocols enables three IC<sub>50</sub> values to be calculated from each cell to give a measure of potency based on resting, inactivated and use-dependent activity.
- Data are in line with published reports (where available)
- QPatch allows high quality data to be produced at a significantly higher throughput compared to the manual patch clamp technique

Compared to IonWorks data, the continual voltage control applied by QPatch enables assessment of compound activity at the resting state and provides information on the kinetics of block

This assay should provide a valuable safety screen for compounds heading towards the clinic, particularly those targeting other voltage-gated sodium channels

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